Purification of poly(A) RNA

User Manual

NucleoTrap® mRNA

August 2010 / Rev. 04

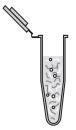
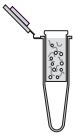
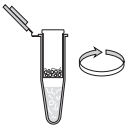
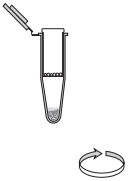
MACHEREY-NAGEL

Purification of poly(A) mRNA

Protocol-at-a-glance (Rev. 04)

Mini / Midi

NucleoTrap® mRNA

1	**Adjust binding conditions**		Proceed from a total RNA **pellet** 100 – 500 µg: 500 µL RM1 500 – 1 000 µg: 1 000 µg RM1 Proceed from a total RNA **solution** 200 – 500 µL: 1 vol RM0
2	**Bind poly(A) RNA**		15 µL Oligo(dT) Latex Beads suspension per 100 µg total RNA 68°C 5 min RT 10 min Invert every 2 min 2,000 x *g* 15 s 11,000 x *g* 2 min
3	**Washing**		Discard supernatant from step 2. 1st wash: 600 µL RM2 Completely resuspend pellet. Transfer Oligo(dT) Latex Beads suspension onto the NucleoTrap® Microfilter. 2nd wash: 500 µL RM3 3rd wash: 500 µL RM3 1st, 2nd, 3rd: 2,000 x *g*, 15 s 11,000 x *g*, 2 min
4	**Dry Oligo(dT) Latex Beads**		11,000 x *g* 1 min
5	**Elute poly(A) RNA**		20 µL RNase-free H_2O per 10 µL Oligo(dT) Latex Beads 68°C 7 min 11,000 x *g* 1 min

MACHEREY-NAGEL GmbH & Co. KG • Neumann-Neander-Str. 6-8 • D-52355 Düren • Germany
Tel.: +49 (0) 24 21 969 270 • www.mn-net.com • e-mail: tech-bio@mn-net.com

Table of contents

1 Components 4
- 1.1 Kit contents 4
- 1.2 Consumables and equipment to be supplied by the user 5
- 1.3 About this User Manual 5

2 Product description 6
- 2.1 The basic principle 6
- 2.2 Kit specifications 6
- 2.3 Pre-isolation of total RNA 7
- 2.4 Handling, preparation, and storage of starting materials 8

3 Storage conditions and preparation of working solutions 9

4 Safety instructions – risk and safety phrases 9

5 Protocol for the isolation of poly(A) RNA 10
- 5.1 Poly(A) RNA isolation from total RNA with NucleoTrap® mRNA 10
- 5.2 Direct purification of poly(A) RNA from cells 13

6 Appendix 14
- 6.1 Troubleshooting 14
- 6.2 Ordering information 16
- 6.3 Product use restriction/warranty 17

1 Components

1.1 Kit contents

	NucleoTrap® mRNA	
	12 preps (Mini)	**12 preps (Midi)**
REF	**740655**	**740656**
NucleoTrap® Oligo(dT) Latex Beads*	480 µL	1800 µL
Lysis Buffer RM0	12 mL	12 mL
Binding Buffer RM1	12 mL	12 mL
Wash Buffer RM2	10 mL	20 mL
Wash Buffer RM3	15 mL	15 mL
RNase-free H_2O	4 mL	8 mL
NucleoTrap® Microfilter (blistered with Microcentrifuge Tubes)	12	12
Microcentrifuge Tubes	24	24
User Manual	1	1

* For preparation of working solutions and storage conditions see section 3.

1.2 Consumables and equipment to be supplied by the user

Consumables

- 1.5 mL microcentrifuge tubes
- Disposable RNase-free pipet tips

Equipment

- Manual pipettors
- Centrifuge
- Vortex mixer
- Thermal heating block
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this User Manual

It is strongly recommended that first-time users of the **NucleoTrap® mRNA** kit read the detailed protocol sections of this User Manual. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at *www.mn-net.com*.

2 Product description

2.1 The basic principle

NucleoTrap® mRNA kits are designed for the isolation and enrichment of poly(A) RNA from pre-isolated total RNA. Most eukaryotic mRNA molecules contain long stretches (about 200 bases) of poly(A) at their 3´ends. Thus most mRNAs can be isolated via their poly(A) tails. The quantity of poly(A) RNA is usually between 1 – 5% of total cellular RNA. The percentage of poly(A) RNA depends on cell type, the growth/physiological state of the cell, and storage conditions of the cell material. Enrichment of poly(A) RNA is highly recommended for the construction of cDNA libraries or demanding blotting procedures for which a reduction in background signals is desirable, for example very low abundant transcripts.

2.2 Kit specifications

- **NucleoTrap® mRNA** kits contain modified latex beads with oligo(dT) nucleotides covalently linked to the surface. Under high-salt conditions, poly(A) RNA will bind to these beads. The resulting A-(dT) hybrids are less stable under lower ionic strength conditions, thus poly(A) RNA can be eluted with water or low salt buffer. For the elution of poly(A) RNA we recommend using the supplied RNase-free H_2O.
- A support protocol contains information for direct purification of poly(A) RNA from cells. In this case, additional equipment and buffers are necessary which are not included in the kit. In general, we recommend purification of poly(A) RNA from total RNA preparations.
- High binding capacity: >5 µg poly(A) RNA / 20 µL Oligo(dT) Latex Beads suspension.
- The **NucleoTrap® mRNA** mini and midi kits contain a 50 mg/mL of Oligo(dT) Latex Beads suspension in 10 mM Tris/HCl, 0.1 M NaCl, 0.1% SDS, 0.05% NaN_3 (pH 7.5).
- Both kits are sufficient for 12 poly(A) RNA preparations.
- Precipitated total RNA pellets as well as total RNA solutions can be used as starting material.
- Purified poly(A) RNA from **NucleoTrap® mRNA** kits is ready for use in all downstream applications.
- Poly(A) RNA is not degraded and without DNA contaminations.
- Each **NucleoTrap® mRNA** mini preparation includes 40 µL Oligo(dT) Latex Beads which allow processing of 200 – 250 µg of total RNA on average and give a maximum yield of 10 µg poly(A) RNA.

- Each **NucleoTrap® mRNA** midi preparation includes 150 µL Oligo(dT) Latex Beads which allow processing of up to 1000 µg of total RNA on average and give a maximum yield of 40 µg poly(A) RNA. Both kits are highly flexible and allow appropriate combination of total RNA and latex bead suspension.

Table 1: Kit specifications at a glance

Parameter	NucleoTrap® mRNA (Mini)	NucleoTrap® mRNA (Midi)
Sample material	Up to 250 µg total RNA	Up to 1 000 µg total RNA
Fragment size	50 b – 20 kb	50 b – 20 kb
Typical yield	Up to 10 µg mRNA	Up to 40 µg mRNA
A_{260}/A_{280}	1.9 – 2.1	1.9 – 2.1
Elution volume	10 – 20 µL	10 – 20 µL
Binding capacity	5 µg poly(A) RNA/ 20 µL Oligo(dT) Latex Beads suspension	5 µg poly(A) RNA/ 20 µL Oligo(dT) Latex Beads suspension
Preparation time	30 min/6 preps	30 min/6 preps

2.3 Pre-isolation of total RNA

For the purification of high quality intact poly(A) RNA, a critical parameter is the quality of the total RNA. Therefore we recommend isolating total RNA first. Further information is given in the following tables:

Table 2: Kits for the isolation of total RNA using NucleoSpin® technology

	NucleoSpin® RNA II (Mini)	NucleoSpin® RNA L (Midi)	NucleoSpin® RNA Plant (Mini)
REF	**740955.20/.50/.250**	**740962.20**	**740949.20/.50/.250**
Sample size	Up to 5 x 10^6 cells Up to 30 mg tissue	Up to 5 x 10^7 cells Up to 200 mg tissue	Up to 100 mg plant tissue or filamentous fungi
Average yield	Up to 70 µg	Up to 400 µg	Up to 70 µg

Table 3: Kits for the parallel isolation of total RNA/DNA/protein		
	NucleoSpin® RNA/Protein (RNA and protein) **(Mini)**	**NucleoSpin® TriPrep*** (RNA, DNA, and protein) **(Mini)**
REF	**740933.10/.50/.250**	**740966.10/.50/.250**
Sample size	Up to 5 x 10^6 cells Up to 30 mg tissue Up to 100 mg plant tissue	Up to 5 x 10^6 cells Up to 30 mg tissue Up to 100 mg plant tissue
Average yield	Up to 70 µg	Up to 70 µg

Table 4: Kits for the isolation of total RNA using NucleoBond® technology		
	NucleoBond® RNA/DNA 80 **(Mini)**	**NucleoBond® RNA/DNA 400** **(Midi)**
REF	**740650**	**740651**
Sample size	Up to 5 x 10^6 eukaryotic cells Up to 0.5 x 10^8 bacterial cells Up to 20 mg tissue	Up to 2 x 10^7 eukaryotic cells Up to 2 x 10^9 bacterial cells Up to 100 mg tissue
Average yield	Up to 70 µg	Up to 400 µg

2.4 Handling, preparation, and storage of starting materials

Eluted total RNA should immediately be put and always kept on ice for optimal stability because almost omnipresent RNases (general lab ware, fingerprints, dust) will degrade RNA. For short-term storage freeze at -20°C, for long-term storage freeze at -70°C.

* DISTRIBUTION AND USE OF NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

3 Storage conditions and preparation of working solutions

Attention:

Buffers RM1, RM2, RM3, and RM0 contain LiCl. Wear gloves and goggles!

- **Oligo(dT) Latex Beads** should be stored at 4°C upon arrival. All other kit components may be stored at 4°C or room temperature (18 – 25°C). Storage at 4°C may cause salt precipitation. In this case buffers should be preheated to 37°C before use until all precipitates are dissolved. The Oligo(dT) Latex Beads settle on the bottom of the tube. To ensure equal distribution, the Oligo(dT) Latex Beads suspension should be vortexed moderately before use.
- All kit components are stable up to one year if stored correctly as described above.

4 Safety instructions – risk and safety phrases

The following components of the **NucleoTrap® mRNA** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Compo-nent	Hazard contents	Hazard symbol
RM0	Lithium chloride	Substance does not have to be specially labeled as hazardous
RM1	Lithium chloride	Substance does not have to be specially labeled as hazardous
RM2	Lithium chloride	Substance does not have to be specially labeled as hazardous
RM3	Lithium chloride	Substance does not have to be specially labeled as hazardous

5 Protocol for the isolation of poly(A) RNA

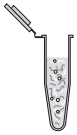
5.1 Poly(A) RNA isolation from total RNA with NucleoTrap® mRNA

1 Adjust binding conditions

RNA pellet

Add **500 µL Buffer RM1** to a pellet which contains **100 – 500 µg total RNA** and **1000 µL Buffer RM1** to a pellet which contains up to **1000 µg** total RNA. Pipette up and down and vortex well in order to guarantee a good resuspension.

+ 500 µL or 1000 µL RM1



RNA solution

To process a **200 – 500 µL total RNA** sample (in water, TE buffer, or usual reaction buffers) add the **same volume of Buffer RM0**.

+ 1 vol RM0

2 Bind poly(A) RNA

+ 15 µL Oligo(dT) Latex Beads Suspension

Resuspend the Oligo(dT) Latex Beads by vortexing. Add **15 µL Oligo(dT) Latex Beads suspension** per **100 µg total RNA**. Mix well and incubate at **68°C** for **5 min**, then incubate at **room temperature** for **10 min** and invert the tube every 2 min during incubation.

68°C 5 min

RT 10 min Invert every 2 min

During incubation at 6 – 8°C the secondary structure of RNA is denatured. Mixing and subsequent incubation at room temperature are important for efficient binding of poly(A) RNA to the Oligo(dT) Latex Beads.

Centrifuge for **15 s** at **2,000 x *g***, then for **2 min** at **11,000 x *g*** in a microcentrifuge tube.

15 s 2,000 x *g*

2 min 11,000 x *g*

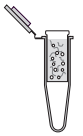
The high-speed centrifugation step is recommended to obtain a tight pellet and to minimize the loss of Oligo(dT) Latex Beads.

3 Washing

1st wash

Discard supernatant and dissolve the Oligo(dT) Latex Beads pellet completely in **600 µL Buffer RM2** by pipetting up and down and vortexing.

Dissolve pellet completely until solution becomes „milky“ and no pellet is visible – this step is important for optimal removal of contaminants such as DNA and rRNA.



+ 600 µL RM2

2,000 x *g*
15 s

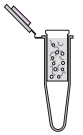
11,000 x *g*
2 min

Transfer the Oligo(dT) Latex Beads suspension into the **NucleoTrap® Microfilter** placed in a Microcentrifuge Tube and centrifuge for **15 s** at **2,000 x *g***, then for **2 min** at **11,000 x *g***. Discard the Microcentrifuge Tube with the flow-through. The Oligo(dT) Latex Beads are retained in the filter insert. Place the NucleoTrap® Microfilter in a fresh Microcentrifuge Tube.

*If more than 500 µg total RNA has been used as starting amount, perform an additional washing step: add **400 µL Buffer RM2** to the Oligo(dT) Latex Beads in the NucleoTrap® Microfilter. Resuspend Oligo(dT) Latex Beads directly on the NucleoTrap® Microfilter by pipetting up and down carefully. Centrifuge for **15 s** at **2,000 x g** and for **2** at **11,000 x g**. Discard flow-through. Avoid puncturing the NucleoTrap® Microfilter!*

2nd wash

Add **500 µL Buffer RM3** to the Oligo(dT) Latex Beads and resuspend them directly in the NucleoTrap® Microfilter by pipetting up and down carefully. Avoid puncturing the NucleoTrap® Microfilter. Centrifuge for **15 s** at **2,000 x *g***, then for **2 min** at **11,000 x *g***. Discard the Microcentrifuge Tube with the flow-through. Place the NucleoTrap® Microfilter in a fresh Microcentrifuge Tube.



+ 500 µL RM3

2,000 x *g*
15 s

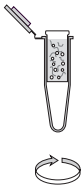
11,000 x *g*
2 min

Resuspend Oligo(dT) Latex Beads completely until the solution becomes „milky“ and no pellet is visible. This step is important for the removal of ribosomal RNA. To make the resuspension of the pellet easier, mark the pellet‘s position after centrifugation, or centrifuge NucleoTrap® Microfilter with identical orientation regarding the position of the lid.

3rd wash

Add **500 µL Buffer RM3** to the Oligo(dT) Latex Beads and resuspend them completely as described in 2nd wash step. Centrifuge for **15 s** at **2,000 x *g***, then for **2 min** at **11,000 x *g***. Discard flow-through and place the NucleoTrap® Microfilter back in the Microcentrifuge Tube.

Dissolve pellet completely until the solution becomes „milky" and no pellet is visible. This step is important for the optimal removal of residual Buffer RM2.



+ 500 µL RM3

2,000 x *g*
15 s

11,000 x *g*
2 min

---

4 Dry Oligo(dT) Latex Beads

Centrifuge the NucleoTrap® Microfilter for **1 min** at **11,000 x *g*** to completely remove the washing buffer. Transfer NucleoTrap® Microfilter to a clean RNase-free 1.5 mL elution tube (not provided).

Residual washing buffer may inhibit subsequent reactions.

11,000 x *g*
1 min

---

5 Elute poly(A) RNA

Add **20 µL prewarmed (68°C) RNase-free H_2O** per **10 µL Oligo(dT) Latex Beads** and resuspend Oligo(dT) Latex Beads completely by pipetting up and down (elution buffer becomes "milky"). Close lids and incubate NucleoTrap® Microfilter at **68°C** for **7 min**. Centrifuge for **1 min** at **11,000 x *g*** and collect eluate.

For higher yields: Repeat the elution step and combine eluates – a second elution step will typically result in a 10 – 20% increased yield but a less concentrated eluate. Transfer combined eluates to a clean 1.5 mL elution tube and store on ice. Subsequent reactions should be performed immediately – if this is not possible, store the eluates at -70°C.

+ 20 µL H_2O RNase-free

68°C
7 min

11,000 x *g*
1 min

5.2 Direct purification of poly(A) RNA from cells

For direct purification of poly(A) RNA from cells additional equipment and buffers are necessary which are **not included in the NucleoTrap® poly(A) RNA kit**. In general, we recommend purification of poly(A) RNA from pre-purified total RNA.

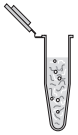
Harvest cells

Precipitate cells by centrifugation (starting material containing approximately 100 µg total RNA should be used, with a maximum of 1 x 10^8 cells.)

500 x *g*
5 min

1 Adjust binding conditions

Add **600 µL Buffer RM0** to the sample. To resuspend the cell pellet, pipette up and down, and vortex well.



+ 600 µL RM0

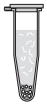
Load lysate onto a **NucleoSpin® Filter** (not included in this kit, see ordering information) and centrifuge for **1 min** at **11,000 x *g***. Add **600 µL RNase-free water** to the clear flow-through and mix well by vortexing.

Optional: The lysate may be passed alternatively ≥ 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.

2 Bind poly(A) RNA

+ 15 µL Oligo(dT) Latex Beads Suspension

Resuspend the Oligo(dT) Latex Beads by vortexing. Add **20 µL Oligo(dT) Latex Beads suspension** per **100 µg total RNA**. Mix well and incubate at **68°C** for **5 min,** then incubate at **room temperature** for **10 min** and invert the tube every 2 min.



68°C
5 min

RT
10 min
Invert every 2 min

During incubation at 68°C the secondary structure of RNA is denatured. Mixing and subsequent incubation at room temperature are important for efficient binding of poly(A) RNA to the beads.

Centrifuge for **15 s** at **2,000 x *g***, then for **5 min** at **11,000 x *g*** in a microcentrifuge tube.

15 s
2,000 x *g*

2 min
>10,000 x *g*

The high-speed centrifugation step is recommended to obtain a tight pellet and to minimize the loss of Oligo(dT) Latex Beads.

Proceed with the standard protocol step 3 section 5.1.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low yield and/or degraded poly(A) RNA	*Washing procedure: Wash Buffer RM3 was not removed completely during centrifugation* • Prolong the subsequent centrifugation step in order to dry NucleoTrap® Microfilter and Oligo(dT) Latex Beads. *Elution procedure – check the following parameters:* • Elution volume too low? • Elution buffer too cold? • Oligo(dT) Latex Beads completely resuspended in elution buffer? *poly(A) RNA binding and integrity* • To avoid insufficient binding of poly(A) RNA, check incubation temperature and time during hybridization. • Check integrity of total RNA preparation on a denaturing agarose gel before enrichment of poly(A) RNA. Fragmented total RNA will yield in less poly(A) RNA. • RNase contamination: clean working place and use RNase-free pipette tips and gloves.
Subsequent reactions failed	*RM3 washing and removal* • Repeat wash step with Buffer RM3. Prolong subsequent centrifugation step in order to dry the NucleoTrap® Microfilter and Oligo(dT) Latex Beads in order to remove any Buffer RM3. *poly(A) RNA quality verified?* • Process positive controls for subsequent reactions. • Check poly(A) RNA quality by gel electrophoresis or blotting experiments with standards.

Problem	Possible cause and suggestions
Direct isolation of poly(A) RNA from cells failed	*Insufficient lysis* • Instead of using Lysis Buffer RM0, alternative buffers for direct poly(A) RNA isolation can be used. For example, 1 M GITC or 0.5 M NaCl with 1% SDS, 5 mM DTT at pH 7 – 8. Keep in mind that low ionic strength conditions destabilize binding of poly(A) RNA to the Oligo(dT) Latex Beads. Avoid using < 0.1 M salt for binding and washing procedures. Always ensure that all reagents are RNase-free.
rRNA contamination	*Total RNA contains approximately 80% of rRNA* • It is difficult to recover poly(A) RNA which is rRNA free with a single Oligo(dT) selection round using the NucleoTrap® mRNA kit. A typical poly(A) isolation with NucleoTrap® mRNA kit yields poly(A) RNA with a reduced rRNA level acceptable for virtually all molecular biological procedures. If lower rRNA levels are desirable, perform a second selection round with the NucleoTrap® mRNA kit.

6.2 Ordering information

Product	REF	Pack of
NucleoTrap® mRNA (mini)	740655	12
NucleoTrap® mRNA (midi)	740656	12
NucleoSpin® Filters	740606	50
NucleoSpin® RNA II	740955.10/.20/.50/.250	10/20/50/250
NucleoSpin® RNA L	740962.20	20
NucleoSpin® RNA Plant	740949.10/.50/.250	10/50/250
NucleoSpin® RNA/Protein	740933.10/.50/.250	10/50/250
NucleoSpin® TriPrep*	740966.10/.50/.250	10/50/250

Visit *www.mn-net.com* for more detailed product information.

* DISTRIBUTION AND USE OF NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

6.3 Product use restriction / warranty

NucleoTrap® mRNA kit components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoTrap® mRNA** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Please contact:
MACHEREY-NAGEL Germany
Tel.: +49 (0) 24 21 969 270
e-mail: TECH-BIO@mn-net.com

Last updated: 12/2006, Rev. 02